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The Pulmonary Localization of Virus-Specific T Lymphocytes Is Governed by the Tissue Tropism of Infection

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ABSTRACT

The migration of pathogen-specific T cells into nonlymphoid tissues, such as the lung, is critical to control peripheral infections. Use of *in vivo* intravascular labeling of leukocytes has allowed for improved discrimination between cells located in the blood from cells present within peripheral tissues, such as the lung. This is particularly important in the lung, which is comprised of an intricate network of blood vessels that harbors a large proportion of the total blood volume at any given time. Recent work has demonstrated that >80% of antigen-specific effector CD8 T cells remain in the pulmonary vasculature following an intratracheal infection with a systemic viral pathogen. However, it remains unclear what proportion of effector CD8 T cells are located within lung tissue following a localized respiratory viral infection. We confirm that most effector and memory CD8 T cells are found in the vasculature after an intranasal infection with the systemic pathogens lymphocytic choriomeningitis virus (LCMV) or vaccinia virus (VACV). In contrast, following pulmonary viral infections with either respiratory syncytial virus (RSV) or influenza A virus (IAV), 80 to 90% of the antigen-specific effector CD8 T cells were located within lung tissue. Similarly, the majority of antigen-specific CD4 T cells were present within lung tissue during a pulmonary viral infection. Furthermore, a greater proportion of gamma interferon-positive (IFN-γ^+) effector CD8 and CD4 T cells were located within lung tissue following a localized respiratory viral infection. Our results indicate that T cells exhibit significantly altered distribution patterns dependent upon the tissue tropism of the infection.

IMPORTANCE

The migration of T cells to nonlymphoid sites, such as the lung, is critical to mediate clearance of viral infections. The highly vascularized lung holds up to 40% of blood, and thus, the T cell response may be a reflection of lymphocytes localized to the pulmonary vasculature instead of lung tissue. We examined the localization of T cell responses within the lung following either a localized or systemic viral infection. We demonstrate that following intranasal infection with a systemic pathogen, most T cells are localized to the pulmonary vasculature. In contrast, T cells are primarily localized to lung tissue following a respiratory viral infection. Our results demonstrate vast differences in the localization of T cell responses within the lung parenchyma between pathogens that can replicate locally versus systemically and that intravascular antibody labeling can be utilized to assess the localization patterns of T cell responses in nonlymphoid organs.

An intricate network of blood vessels is associated with the bronchial tree and alveolar sacs of the lung (1). The vascular network is necessary for respiratory function as well as for the trafficking of leukocytes into the lung during infection (2). Leukocytes that remain in the vasculature can be detected by intravenous (i.v.) administration of a specific antibody prior to perfusion and tissue isolation (3–5). Recent work has shown that 80 to 95% of T cell receptor (TCR) transgenic effector and memory CD8 T cells are confined to the pulmonary vasculature following an intratracheal lymphocytic choriomeningitis virus (LCMV) infection despite extensive lung perfusion (4). Importantly, LCMV disseminates systemically even after an intratracheal or intranasal (i.n.) infection. Therefore, it remains unclear what proportion of T cells isolated from a perfused lung are within the lung tissue versus the pulmonary vasculature following an intranasal inoculation with a virus causing a localized respiratory infection.

CD8 T cells play a critical role in mediating clearance of acute localized respiratory viral infections such as those caused by respiratory syncytial virus (RSV) and influenza A virus (IAV) (6, 7). Due to the critical role CD8 T cell responses play in mediating the clearance of respiratory viral infections, we sought to determine whether the majority of endogenous virus-specific CD8 T cells were located within lung tissue following a localized pulmonary viral infection. In contrast to systemic infections, our results demonstrate that 80 to 90% of endogenous virus-specific effector and memory CD8 T cells are located within lung tissue following a localized pulmonary viral infection. Furthermore, using cytokine reporter mice, we show that gamma interferon-positive (IFN-γ^+) endogenous effector CD8 T cells are highly enriched within lung tissue following a respiratory viral infection compared to a systemic infection. These data indicate that the tissue tropism of a virus significantly impacts the localization pattern of virus-specific effector and memory CD8 T cells.

MATERIALS AND METHODS

Mice, adoptive transfers, and infection. C57BL/6NCr mice (6 to 8 weeks old) were obtained from the National Cancer Institute (Frederick, MD).

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For adoptive transfers, 1×10^3 naive P14 CD90.1/CD90.2 cells were injected i.v. 1 day prior to infection with one of the following: (i) 2×10^5 PFU LCMV Armstrong intraperitoneally (i.p.); (ii) 5×10^5 PFU LCMV Armstrong i.n.; or (iii) 230 50% tissue culture infective units ($TCIU_{50}$) of IAV strain A/Puerto Rico/8/1934 (PR8) expressing GP_{33-41} (i.e., amino acids 33 to 41 from LCMV glycoprotein) (IAV-GP_{33}) (i.n.). BALB/cAnNCr mice (6 to 8 weeks old) were infected i.n. with 5×10^5 PFU LCMV Armstrong, 5×10^3 PFU vaccinia virus (VACV) strain Western Reserve, 110 to 150 $TCIU_{50}$ IAV PR8 or 1.6×10^6 RSV A2. *Ifng/CD90.1* knock-in mice (8) obtained from Stanley Perlman (University of Iowa) with permission from Casey Weaver (University of Alabama at Birmingham) were infected i.n. with either 5×10^5 PFU LCMV Armstrong or 110 $TCIU_{50}$ IAV PR8. All experimental procedures involving mice were approved by the University of Iowa Animal Care and Use Committee.

Intravascular antibody labeling and tissue processing. One microgram of CD90.2-PE (CD90.2 labeled with phycoerythrin [PE]) (clone 53-2.1) antibody was injected via the tail vein i.v. 3 min prior to euthanasia, and cells from various tissues were subsequently prepared as previously described (4).

Tetramer and antibody staining. Cells were incubated with a single tetramer, either LCMV GP_{33-41} or IAV $NP_{366-374}$ (i.e., amino acids 366 to 374 from nucleoprotein) for C57BL/6 mice or LCMV $NP_{118-126}$, VACV $F2_{26-34}$, IAV $HA_{518-526}$ (i.e., amino acids 518 to 526 from hemagglutinin), or RSV $M2_{82-90}$ (i.e., amino acids 82 to 90 from M2) for BALB/c mice (obtained from the NIH Tetramer Facility) and subsequently stained for extracellular expression of CD90.2 (clone 53-2.1), CD8 (clone 53-6.7), CD4 (clone RM4-5), CD11a (clone M17/4), and CD49d (clone R1-2) and intracellular expression of Foxp3 (clone FJK-16S; all antibodies obtained from BioLegend, San Diego, CA) as previously described (9).

Plaque assay. Lungs were harvested on day 4 postinfection (p.i.). Whole lungs were homogenized, and supernatants were subsequently collected and flash-frozen. For plaque assay, supernatants were thawed and plated on BSC40 cells for VACV-infected lungs, MDCK cells for IAV-infected lungs, or Vero cells for either LCMV- or RSV-infected lungs as previously described (10–12). Briefly, samples were diluted in serum-free medium and plated on the appropriate cell line with rocking for 1 to 1.5 h. For LCMV and RSV, cells were subsequently overlaid with a 1:1 ratio of serum-containing medium and 1% agar. On day 3 for LCMV or day 5 for RSV, the plaques were stained with a 1:1 ratio of medium and 1% agar containing 0.01% neutral red. The plaques were visually quantified 24 h later. For IAV plaque assays, infected MDCK cells were overlaid with a 1:1 ratio of serum-containing medium and 1.6% agar. On day 3 p.i., the agar plug was removed, and the cells were fixed with 70% ethanol. The IAV-infected MDCK cells were subsequently stained with crystal violet and rinsed with warm water, and plaques were immediately counted. For VACV plaque assays, serum-containing medium was added to the VACV-infected BSC40 cells. On day 2 p.i., the infected cells were fixed with 7% formaldehyde, stained with crystal violet, and rinsed with warm water. VACV plaques were subsequently counted for virus quantification.

Statistical analysis. Data were compiled, and statistical analysis was calculated by performing a one-way analysis of variance (ANOVA) with a Tukey's posttest for more than two groups or a Student's unpaired *t* test for two groups in Prism software (GraphPad Software, San Diego, CA). Values that were significantly different are indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

RESULTS

Virus-specific CD8 T cells preferentially localize to the lung following RSV infection. Intravascular staining has been utilized in several recent studies to discriminate between leukocytes in lung tissue versus pulmonary vasculature and has been previously shown not to stain perivascular cells (3–5). To determine the proportion of endogenous antigen-specific CD8 T cells that have entered lung tissue following a localized pulmonary viral infection, RSV-infected mice were injected intravenously with an anti-CD90.2 antibody (Fig. 1A) to specifically label all T cells in the peripheral blood. In addition, T cells localized within either secondary lymphoid tissues or bronchoalveolar lavage (BAL) fluid were protected from intravascular labeling with antibody (Fig. 1A). Antibody specific to CD90.2 was chosen to avoid any potential issues with the known downregulation of CD8α on murine CD8 T cells following antigen stimulation (13). The frequency of total CD8 T cells not labeled with antibody, representing cells in lung tissue, increased following RSV infection, peaking at day 8 p.i. (Fig. 1B). Similarly, the frequency of unlabeled $CD11a^{hi}$ $CD8^{lo}$ T cells, used to define antigen-specific cells as previously described (13, 14), and $M2_{82-90}$ tetramer-positive CD8 T cells also peaked in lung tissue at day 8 p.i. The frequency of total CD8 T cells that remained unlabeled in the lung decreased significantly by day 30 p.i., consistent with contraction of the T cell response. However, the frequency of unlabeled antigen-specific CD8 T cells located within lung tissue remained relatively stable and similar to the peak of the response. Overall, approximately 85% of tetramer-positive CD8 T cells in the perfused lung remained in the lung tissue for up to 30 days following RSV infection. Furthermore, >95% of antigen-specific CD8 T cells that expressed either one of the tissue-resident markers CD69 and CD103 (15–17) were located within lung tissue at day 30 p.i. (Fig. 1D). However, only 40% or 50% of antigen-specific CD8 T cells within lung tissue expressed either CD69 or CD103, respectively, 30 days following RSV infection (Fig. 1E). In contrast to the antigen-specific cells, the frequency of unlabeled $CD11a^{lo}$ $CD8^{hi}$ naive T cells remained relatively constant, suggesting that naive T cells do not preferentially enter the lung following infection.

Similar to CD8 T cells, conventional CD4 T cells shared the same localization pattern within the lung parenchyma following RSV infection (Fig. 1C). The frequency of CD4 T cells that were unlabeled within lung tissue increased following infection, peaking at day 8 p.i., but decreased over time in correlation with contraction of the T cell response. However, approximately 70% of antigen-experienced $CD11a^{hi}$ $CD49d^{+}$ CD4 T cells (18) remained localized to lung tissue up to 30 days following RSV infection, whereas the frequency of unlabeled naive $CD11a^{lo}$ $CD49d^{-}$ CD4 T cells was unchanged. Regulatory $Foxp3^{+}$ CD4 T cells exhibited a pattern of localization within lung tissue that was similar to that of antigen-experienced CD4 T cells, peaking at day 8 p.i. and decreasing over time. This suggests that regulatory CD4 T cells localize within lung tissue coincident with the effector T cell response to suppress the adaptive immune response.

Increased frequency of effector T cells in lung tissue following pulmonary infections. To determine whether a localized pulmonary infection would induce the preferential migration of effector CD8 T cells into lung tissue compared to a systemic infection, BALB/c mice were infected i.n. with IAV or RSV, both of which cause infections that are localized to the lung, or with LCMV or VACV, both of which cause systemic infections. On day 8 following either IAV or RSV infection, there was a significant ($P < 0.01$) increase in the frequency of unlabeled CD8 T cells located in lung tissue compared to intranasal infection with the systemic pathogens LCMV and VACV (Fig. 2A). In addition, there was a significantly ($P < 0.01$) increased frequency of unlabeled antigen-specific tetramer-positive CD8 T cells in lung tissue on day 8 following IAV and RSV infection than after either LCMV or VACV infection (Fig. 2B). Similarly, following either IAV or RSV infection at day 8 p.i., there was a significantly ($P < 0.01$)

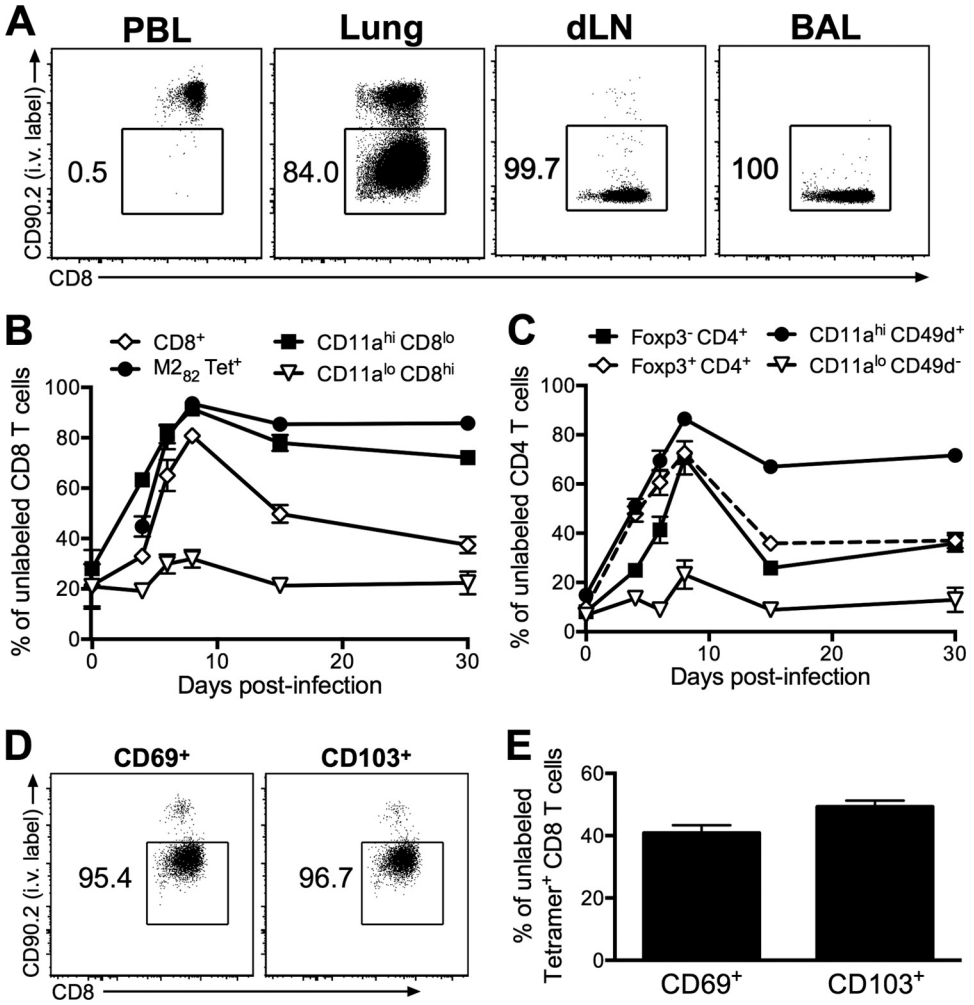

FIG 1 Preferential recruitment of antigen-specific CD8 T cells to the lung following pulmonary infection. (A) Representative dot plots of labeled T cells following i.v. anti-CD90.2 antibody administration. The numbers in the graphs are the frequencies of unlabeled CD8 T cells on day 8 after RSV infection. PBL, peripheral blood leukocytes; dLN, draining lymph nodes; BAL, bronchoalveolar lavage fluid. (B and C) Frequencies of unlabeled CD8 T cell (B) or CD4 T cell (C) populations at multiple time points following RSV infection of BALB/c mice ($n = 8$). (D) Concatenated dot plots of the frequency of unlabeled $CD69^+$ or $CD103^+$ $M2_{82\text{-}90}$ tetramer-positive (Tet^+) CD8 T cells at day 30 p.i. (E) Frequency of unlabeled $M2_{82\text{-}90}$ tetramer-positive CD8 T cells that are either $CD69^+$ or $CD103^+$ at day 30 p.i. Combined results are shown from two independent experiments.

increased frequency of antigen-specific $CD11a^{hi}$ $CD8^{lo}$ T cells that were unlabeled in lung tissue compared to either LCMV or VACV (Fig. 2C). However, there was no difference in the frequency of unlabeled $CD11a^{lo}$ $CD8^{hi}$ naive T cells in lung tissue following any infection (Fig. 2D). These data indicate that antigen-specific CD8 T cells preferentially enter lung tissue and are not simply trapped within the pulmonary vasculature following a viral infection restricted primarily to the respiratory tract.

The distribution of CD4 T cells following systemic and localized pulmonary infections in BALB/c mice was also assessed by intravascular labeling. Recent work by Turner et al. indicates that tissue-resident memory CD4 T cells primarily reside at locations determined by the initial site of infection and demonstrates that the majority of memory CD4 T cells are localized within lung tissue following IAV infection (19). We find that similar to CD8 T cells, conventional $Foxp3^-$, regulatory $Foxp3^+$, and antigen-specific $CD11a^{hi}$ $CD49d^+$ CD4 T cells were preferentially localized within lung tissue following either IAV or RSV infection compared to either LCMV or VACV infection at day 8 p.i. (Fig. 3A to C). Similar to $CD11a^{lo}$ $CD8^{hi}$ naive T cells, there was no difference in the frequency of unlabeled $CD11a^{lo}$ $CD49d^-$ CD4 T cells between infections (Fig. 3D), indicating that naive CD4 T cells were not preferentially recruited to the site of infection. These data suggest that similar to antigen-specific CD8 T cells, activated CD4 T cells preferentially migrate into the lung following a localized pulmonary infection. Importantly, these differences in CD4 and CD8 T cell migration between localized and systemic viral respiratory infection were not due to deficient virus replication in lungs (Fig. 4). Following i.n. infection, lung viral titers were similar for LCMV, VACV, IAV, and RSV (Fig. 4). Furthermore, there was increased virus in LCMV- and VACV-infected lungs following i.n. infections compared to i.p. infections.

To determine whether monoclonal TCR transgenic CD8 T cells exhibit localization patterns similar to those of endogenous polyclonal T cells, we adoptively transferred 1×10^3 LCMV GP_{33}-specific P14 CD8 T cells into naive C57BL/6 mice and challenged

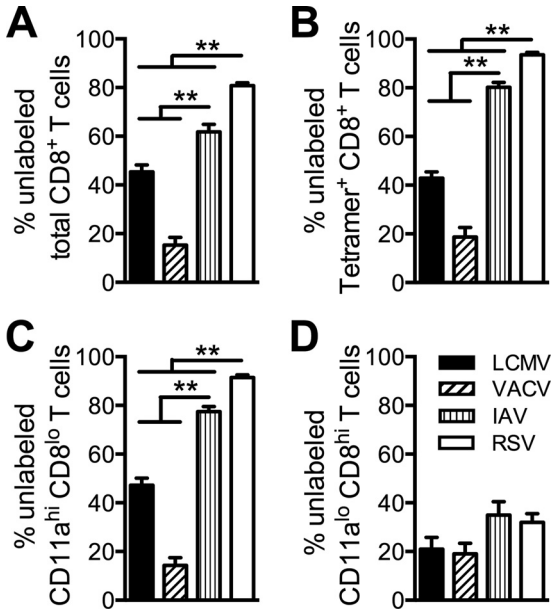

FIG 2 Increased lung localization of antigen-specific CD8 T cells following a restricted pulmonary infection. On day 8 p.i., cells were isolated from the lung following i.v. labeling in BALB/c mice. (A to D) Frequencies of unlabeled total (A), tetramer-positive (B), CD11a^{hi} CD8^{lo} (C), and CD11a^{lo} CD8^{hi} (D) T cells following LCMV, VACV, IAV, and RSV infection. A one-way ANOVA with a Tukey's posttest was performed. Values that are significantly different (**, $P < 0.01$) are indicated by bars and two asterisks. Data are combined from two independent experiments ($n = 8$, where n is the number of mice from both experiments combined).

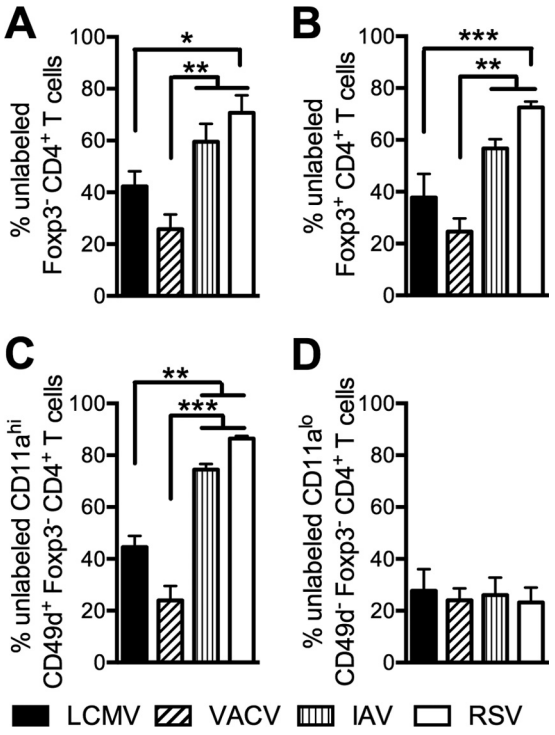

FIG 3 CD4 T cells are preferentially localized to the lung following a local pulmonary infection. Lymphocytes were isolated from the lung on day 8 p.i. following i.v. labeling in BALB/c mice. (A to D) Frequencies of unlabeled conventional Foxp3$^-$ (A), regulatory Foxp3$^+$ (B), CD11a^{hi} CD49d$^+$ (C), and CD11a^{lo} CD49d$^-$ CD4 (D) T cells following LCMV, VACV, IAV, and RSV infection. A one-way ANOVA with a Tukey's posttest was performed. Values that are significantly different are indicated by bars and asterisks as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Combined results from two independent experiments are shown ($n = 8$).

them with LCMV i.p., LCMV i.n., or recombinant IAV-GP$_{33}$ i.n. On day 8 p.i., a significantly ($P < 0.001$) higher frequency of P14 CD8 T cells were located within lung tissue following an IAV-GP$_{33}$ infection compared to either i.p. or i.n. LCMV infections (Fig. 5A). Furthermore, using the LCMV clone 13 strain, which has an increased capacity to disseminate compared to LCMV Armstrong (20, 21), a similar localization pattern of P14 cells in the lung was observed following i.n. infection (Fig. 5B).

When assessing the endogenous T cell response in the recipient mice, a higher frequency of total CD8 T cells was localized to lung tissue following challenge with IAV-GP$_{33}$ compared to either route of LCMV infection (Fig. 5C), similar to the results observed for BALB/c mice. Furthermore, a significantly higher proportion of endogenous GP$_{33-41}$ tetramer-specific CD8 T cells were located within lung tissue following a localized IAV-GP$_{33}$ infection (Fig. 5D). Taken together, our data demonstrate that the localization pattern of antigen-specific T cells is governed by both the route of infection and tissue tropism of the pathogen.

The majority of IFN-γ$^+$ effector CD8 T cells are located within the lung following a respiratory virus infection. Our data thus far have indicated that there is an increased frequency of antigen-specific T cells within the lung tissue following a localized pulmonary infection. However, we further sought to determine whether there were functional differences between CD8 T cells recruited into the lung compared to cells in the peripheral blood following a localized respiratory tract infection versus a systemic infection. To address this question, we infected IFN-γ reporter mice that express CD90.1 following induction of *Ifng* gene transcription (8) with either LCMV or IAV i.n. Following infection with either virus, a large portion of tetramer-positive CD8 T cells were also CD90.1$^+$, indicating that these cells had recently produced IFN-γ (Fig. 6A). To compare CD90.1 expression in the different infections, values were normalized by determining the ratio of the percentage of CD90.1$^+$ unlabeled tetramer-positive CD8 T cells to the percentage of CD90.1$^+$ labeled tetramer-positive CD8 T cells. There was an approximately 2-fold increase in CD90.1/IFN-γ$^+$, tetramer-positive CD8 T cells ($P < 0.001$) local-

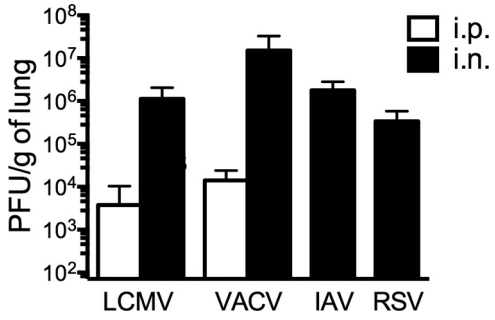

FIG 4 Virus replication in the respiratory tract following localized or systemic infections. Viral titers were quantified in the lungs at day 4 following either LCMV, VACV, IAV, or RSV infection by plaque assay. LCMV and VACV were administered i.p. and i.n. Combined results from two independent experiments are shown ($n = 7$ or 8).

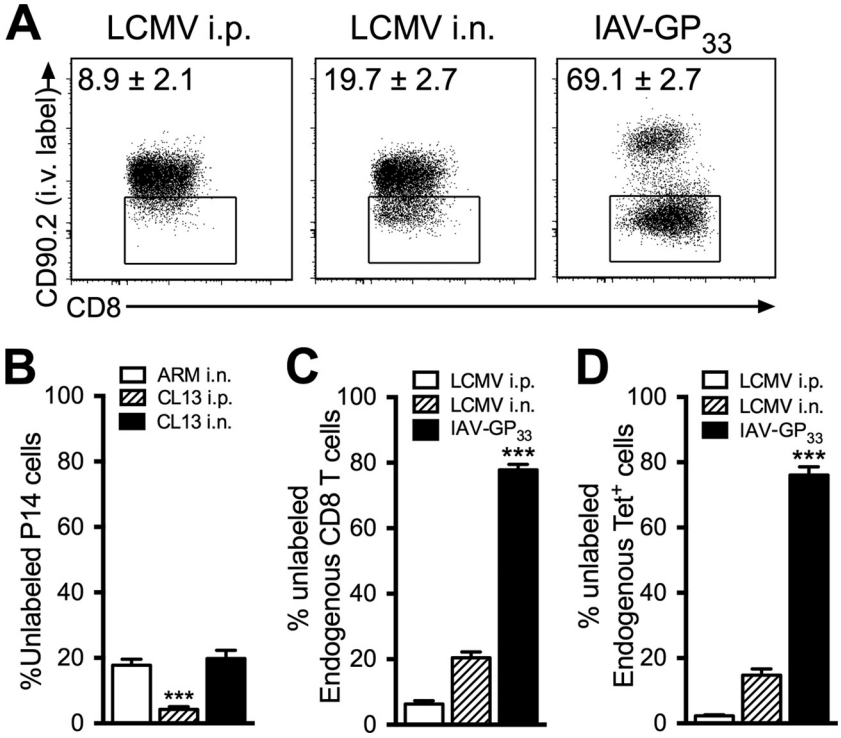

FIG 5 Increased frequency of antigen-specific CD8 T cells confined to lung tissue following localized respiratory viral infection. P14 CD8 T cells were adoptively transferred i.v. into C57BL/6 mice and infected the following day with LCMV Armstrong i.p. or i.n. or IAV-GP_{33} i.n. (A) Concatenated dot plots depicting the frequency of CD90.2 unlabeled CD8 T cells of P14 cells in the lung at day 8 p.i. The numbers in the graphs represent the mean percentages of CD90.2 P14 cells that are i.v. label negative ± standard errors of the means (SEM) for the groups. (B) Frequency of unlabeled P14 cells on day 8 in the lung following infection with either LCMV Armstrong (given i.n.) or clone 13 (CL13) (given i.n. or i.p.). (C and D) Frequencies of unlabeled endogenous CD8 T cells (C) and GP_{33-41} tetramer-positive endogenous CD8 T cells (D). Values that are significantly different ($P < 0.001$) from the values for the other experimental groups are indicated by three asterisks. Combined results from two independent experiments are shown ($n = 8$).

ized to lung tissue following IAV infection compared to LCMV infection (Fig. 6B). Furthermore, the geometric mean fluorescence intensity (gMFI) of CD90.1 expression was significantly ($P < 0.01$) increased in the unlabeled tetramer-positive CD8 T cells following IAV infection than in either the labeled IAV-specific CD8 T cells or LCMV-specific CD8 T cells (Fig. 6C). Similar to CD8 T cells, there was a significantly increased proportion of antigen-experienced CD4 T cells ($P < 0.01$) localized to lung tissue following a localized IAV infection compared to LCMV infection (Fig. 6D). Furthermore, the antigen-experienced CD4 T cells within the lung tissue had a higher gMFI of CD90.1 compared to cells in the pulmonary vasculature following IAV infection and not LCMV infection (Fig. 6E). These data demonstrate that IFN-γ^+ effector CD8 and CD4 T cells are enriched within lung tissue following a pulmonary viral infection compared to a systemic viral infection.

DISCUSSION

Previous work has demonstrated that 80 to 95% of effector and memory TCR transgenic CD8 T cells are confined to the pulmonary vasculature following an intratracheal challenge with the systemic pathogen LCMV (4). Similarly, we demonstrate following an intranasal infection with the systemic pathogen LCMV or VACV that the majority of CD8 T cells remain in the pulmonary vasculature of the lung. In contrast, challenge with either RSV or IAV, both of which replicate only in the respiratory tract, induce the preferential migration of antigen-specific effector CD8 T cells into lung tissue. This is important, as CD8 T cells play a critical role in mediating clearance of respiratory viral infections such as those caused by RSV and IAV (6, 7, 22, 23). In addition, we find that the majority of IFN-γ^+ effector CD8 T cells are located within the lung following a localized respiratory infection compared to a systemic infection. This suggests that there is a greater proportion of fully differentiated CD8 T cells capable of exerting effector functions located within lung tissue following a localized respiratory infection where these cells play a critical role in mediating viral clearance.

We also observed that the frequency of RSV-specific CD8 T cells located within lung tissue remains relatively high (~85%) through day 30 p.i. Expression of CD69 and CD103 has been used to identify tissue-resident memory CD8 T cells that have been shown to form stable long-lasting populations in the lung tissue following respiratory viral infections (24–27). Up to 40% or 50% of the antigen-specific CD8 T cells expressed either CD69 or CD103, respectively, and >95% of these cells were unlabeled, suggesting that they are tissue-resident memory T cells. The role of tissue-resident memory T cells has not been extensively examined in the lung environment following RSV infection. However, studies have indicated that tissue-resident memory T cells correlate with local protective immunity during either Sendai virus or influenza virus infection (28, 29). Thus, it is likely that the tissue-resident memory CD8 T cells following RSV infection contribute to protection against secondary infections. In addition, this

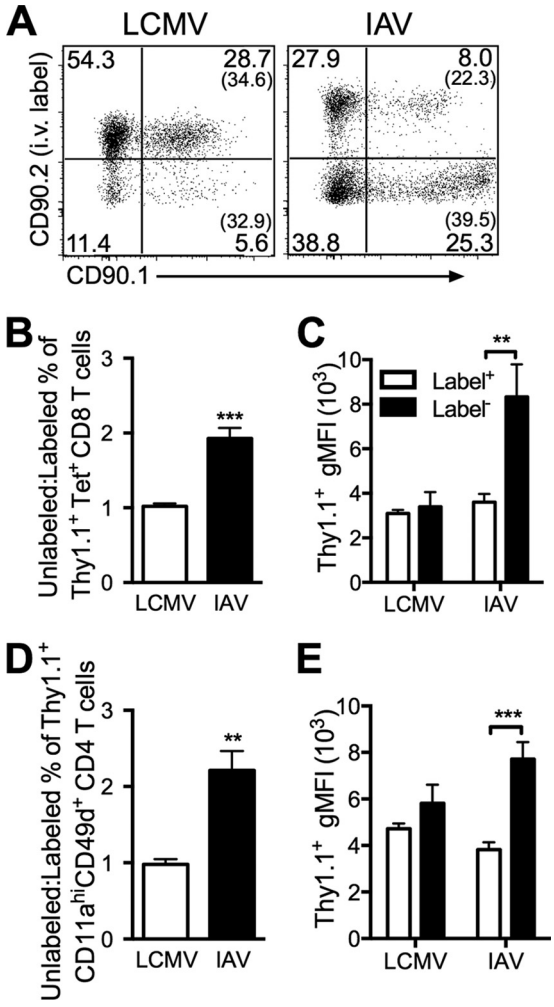

FIG 6 Increased proportion of virus-specific CD8 T cells produce IFN-γ in lung tissue following a localized pulmonary infection. *Ifng/CD90.1* knock-in mice were infected with either LCMV or IAV i.n. and harvested 8 days later. (A) Representative dot plots of intravascular staining for CD90.2 and CD90.1. The numbers in parentheses in the graphs represent the frequencies of CD90.1$^+$ (IFN-γ$^+$) CD8 T cells of the total unlabeled or labeled tetramer-positive CD8 T cell populations. (B and D) Ratio of the percentage of unlabeled CD90.1$^+$ T cells to the percentage of labeled CD90.1$^+$ T cells for tetramer-positive CD8 T cells (B) and CD11a^{hi} CD49d$^+$ CD4 T cells (D). (C and E) Geometric MFI (gMFI) for CD90.1$^+$ staining in the labeled and unlabeled tetramer-positive CD8 T cell populations (C) and CD11a^{hi} CD49d$^+$ CD4 T cells (E). A Student's unpaired *t* test (B and D) or a two-way ANOVA with a Bonferroni's posttest (C and E) was performed ($n = 5$). Values that are significantly different are indicated by bars and asterisks as follows: **, $P < 0.01$; ***, $P < 0.001$. Combined results from two independent experiments are shown.

unique subset of memory cells has been characterized and shown to contribute to local immunity in the skin, brain, vaginal tract, and intestinal epithelium (17, 30–33).

Similar results were observed for the CD4 T cell response. The majority of antigen-experienced effector and memory CD4 T cells were located within lung tissue following localized respiratory infections caused by either RSV or IAV compared to a significantly reduced frequency following infection with systemic pathogens. These results are in agreement with recent work by Turner et al. which shows that most of the effector CD4 T cells are located within lung tissue following IAV infection (19). In addition, lung niches permit the formation and maintenance of IAV-specific, tissue-resident memory CD4 T cells. We also find that following RSV infection, the majority of antigen-experienced CD4 T cells remain in lung tissue for up to 30 days.

Our findings indicate the distinct localization patterns of effector T cells within lung tissue following systemic versus localized respiratory viral infections. Our results highlight the need to accurately assess the total number of antigen-specific T cells within lung tissue following a pulmonary infection with a pathogen capable of replicating systemically. In contrast, the majority of antigen-specific T cells are found within the lung tissue following infection with a pathogen that replicates primarily in the respiratory tract. This lessens the need to take measures to discriminate the location of antigen-specific T cells following a localized respiratory viral infection. Overall, our study indicates that i.v. labeling of T cells using a CD90-specific antibody is a straightforward method to distinguish both CD4 and CD8 T cells in peripheral blood from cells in the lung and that virus-specific CD8 T cells rapidly enter lung tissue following a localized respiratory viral infection.

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We declare that we have no financial conflicts of interest.

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